A Pilot Study of Resting-State Functional Connectivity in Depressed Adolescents with and without Histories of Suicide Attempts

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Suicide is one of the leading causes of death among adolescents and is often associated with major depressive disorder (MDD). Research examining the underlying neurobiology of MDD has found abnormal connectivity within the fronto-limbic regions of the brain. Research on the neural circuitry of depression and suicide is warranted because of the high prevalence and incidence of these problems among young people and because of the ongoing brain development in adolescence. This pilot study used resting-state functional magnetic resonance imaging to compare brain connectivity between three groups: adolescents with MDD and a history of suicide attempts (MDD/ SA) (n = 4), adolescents with MDD and no history of suicide attempts (NSA) (n = 8), and healthy controls (n = 9). Although the results were not significant, they did suggest that the MDD/SA group had lower connectivity among fronto-limbic regions than controls (lateral prefrontal cortex, subgenual anterior cingulate cortex (ACC), and supragenual ACC) and also lower connectivity than both the NSA group and controls among certain regions (subgenual ACC, rostral ACC, amygdala, and nucleus accumbens). Though only trending toward significance, the reduced connectivity in this group may suggest possible impairments in interpreting emotions and regulating emotional responses. Further research in this area can provide important information for the development of appropriate treatment strategies that can target these deficits and thus, provide more effective interventions.

Adolescence is a developmental period which is often associated with the onset of mental disorders. Major depressive disorder (MDD) has received considerable attention due to the prevalence and incidence of this disorder among this population (Lewinsohn, Rohde, & Seeley, 1998). According to the Diagnostic and Statistical Manual of Mental Disorders (4th ed., text rev.; DSM-IV-TR; American Psychiatric Association, 2000), MDD is characterized by at least a two week period consisting of either depressed mood or loss of interest or pleasure. Additionally, individuals must also have at least 4 other symptoms such as insomnia/hypersomnia, fatigue, and feelings of worthlessness. Because of the association of MDD with suicide, research within this field is warranted as it is the third leading cause of death in adolescents (American Academy of Child and Adolescent Psychiatry, 2008). Such research can be used to further the advancement of intervention strategies so that they may be more effective. A way to study adolescent depression and suicide in terms of their neurobiology is through the use of neuroimaging techniques.Specifically, the purpose of this study is to use resting-state functional magnetic resonance imaging to explore the connectivity differences between adolescent MDD patients who have attempted suicide and MDD patients without suicide attempts and healthy controls.

Neuroimaging, including magnetic resonance imaging(MRI) and functional magnetic resonance imaging (fMRI), are research practices that have provided us with great discoveries such as the course of childhood and adolescent brain development (Gieddetal.,1999;Clare Kellyetal.,2009). MRI is a relatively new technique that uses a strong magnetic field and radio frequency fields to align and then alter the alignment of atomic nuclei to produce a change that can be detected by the scanner

(Hendee & Morgan, 1984). Because it does not require exposure to radioactive materials, this procedure is used more routinely for more sustained periods of time and while children are still undergoing development. This neuroimaging method allows researchers and clinicians to acquire information regarding the structure of the brain. fMRI, the method used in this study, is similar to that of MRI except that it records neural activityin the brain by using the blood-oxygen-level-dependent (BOLD) response. Studies using these neuroimaging methods have begun to show structural and functional abnormalities within the brain of depressed adolescents as well as those who attempt suicide.

Evidence for Fronto-LimbicAbnormalities

Research has been conducted to reveal neurobiological differences between MDD patients and healthy controls. These studies assist in providing a base for the exploration of these differences in MDD patients with histories of suicide. In particular, abnormalities within the fronto-limbic region of the brain, including the anterior cingulate cortex(ACC), have been found to be implicated in MDD. Bush, Luu, and Posner (2000) illustrate the idea of a cognitive and affective division of the ACC. The cognitive division is believed to be responsible for various aspects of executive functioning including motivation, response selection, and error detection (Bush et al.,2000).This includes connections between the dorsal region of the ACC and other frontal and limbic regions of the brain such as the lateral prefrontal cortex(PFC). In contrast, Bush and colleagues (2000) explain the affective division as being involved in the regulation of emotional responses, and the appraisal of motivational and emotional information. The affective division includes connections between the ventral region of the ACC and the amygdala, nucleus accumbens, insula, hippocampus, and orbitofrontal cortex(OFC)(Bush et al., 2000). Deficiencies in the connectivity among these regions may underlie the emotional and behavioral difficulties associated with MDD and suicide.

Indeed, post-mortem examinations of suicide victims provide support for fronto-limbic dysregulation. Honer et al.(1999) found decreased myelin basic protein immunoreactivity in the anterior frontal cortex of both MDD and schizophrenia patients who died by suicide, which may suggest impairment in neurotransmission. Furthermore, Torres-Platas and colleagues (2011) found white matter abnormalities in the ACC among depressed suicides compared to sudden-death controls, which also suggests a deficit in neural communication. Although these studies examine what could possibly be the effect of suicide, they provide direction in examining possible precursors to suicidal behaviors.

As reviewed by Lorenzetti et al. (2009), studies have found volumetric abnormalities among adults with MDD within regions involved in the experience of emotion and emotion regulation including decreased hippocampus, OFC, and subgenual ACC and dynamic volume changes of the amygdala. A recent study by Malykhin et al. (2012) also found decreased ACC volumes and increased amygdala among depressed adults.Furthermore,resting-state fMRI studies of adults withMDD have found abnormal connectivity within fronto-limbic brain regions including the dorsal ACC,thalamus,and amygdala (Grecius et al.,2008).Researchers have also examined connectivity differences in the context of visual stimuli. Delvecchio et al. (2012) conducted a metaanalysis of twenty studies which used facial processing paradigms and fMRI. MDD patients showed increased limbic activation in response to all stimuli, decreased sensorimotor activation in response to fearful stimuli, and decreased right pulvinar thalamus activity in response to happy stimuli, implicating these regions in the processing of emotional stimuli (Delvecchio et al., 2012).

Less research has been conducted using adolescent samples with MDD; however, Cullen and colleagues (2010)used diffusion tensor imaging(DTI)to find decreased white matter integrity within fronto-limbic regions of the brain including the subgenual ACC in particular. Using resting-state fMRI data from the same sample,Cullen et al.(2009)found decreased connectivity between the subgenual ACC and supragenual ACC, right medial frontal cortex, left superior and inferior frontal cortex, and insular cortex. Connectivity differences involving the subgenual-ACC network is believed to be related to the emotion dysregulation present among depressed adolescents (Cullen et al.,2009). During the administration of attention and cognitive control tasks, an fMRI study by Halari et al. (2009) found that adolescents with MDD had decreased connectivity in the right dorsolateral and inferior PFC and dorsal and ventral ACC. Taken together, these findings support that there are abnormalities in the connectivity

of this region among MDD patients that may underlie cognitive and emotional deficits.

Fronto-limbic abnormalities in both structure and connectivity are also evident in studies examining suicidal and non-suicidal subjects and healthy controls. Although findings are conflicted between whether or not these structures are larger or smaller in suicidal patients compared to controls,studies have found structural abnormalities in the orbitofrontalcortex, amygdala,hippocampus, caudate, and regions of the ACC among suicidal patients and patients at high risk for suicide(Monkul et al., 2007; Spoletini et al., 2011; Wagner et al., 2011). Further research has found periventricular white matter hyperintensities among child, adolescent, young adult, and elderly MDD patients with histories of suicide attempts compared with MDD patients without such histories (Ehrlich et al.,2004;2005; Ahearn et al.,2001). Using DTI, Jia et al. (2010) found decreased white matter microstructure among MDD patients with suicide attempts within the right frontal lobe compared to controls, right lentiform nucleus compared to depressed non-attempters, and left anterior limb of the internal capsule in contrast to both comparison groups. The above findings of such structural differences among suicidal individuals supports the hypothesis that dysregulation within the cognitive and affective divisions posited by Bush, Luu, and Posner(2000).

Evidence from positron emission tomography (PET)and fMRI studies demonstrates that suicidal patients may exhibit anomalous functional connectivity in key fronto-limbic brain circuitry. Oquendo et al.(2003) used PET and found lower prefrontal cortex activity among depressed patients who made high-lethality suicide attempts compared to those with low-lethality. Using fMRI, Marchand et al. (2012) found that those who had histories of self-harm behaviors, all but one of which being suicide attempts, had a different striatal network compared to depressed patients without self-harm behaviors and also patients with suicide ideation. This network includes the left temporal and inferior parietal lobule regions, motor/sensory cortical regions, and right posterior cortical midline structure (CMS),which includes the ACC. In contrast, an fMRI study by Pan et al.(2011) examined connectivity during a response inhibition task and found no differences between controls and depressed adolescents with histories of suicide. Other than these studies, research exploring the functional abnormalities that may underlie suicidal behaviors among adolescents islimited.

Hypotheses

The aim of this exploratory study is to expand the knowledge regarding the neural circuitry of suicide by examining the resting-state connectivity of depressed adolescents with histories of suicide attempts compared to depressed adolescents without histories of suicide attempts and healthy controls. Due to the support from previous research for populations diagnosed with MDD, this study will focus on possible fronto-limbic abnormalities. The subgenual ACC is of particular interest because of its apparent dysregulation in previous studies as well as its involvement in emotion regulation (see Bush, Luu, & Posner, 2000 for review).Taking together previous research on adolescent MDD and suicide and the theory reviewed by Bush, Luu, and Posner(2000), we hypothesized that the connectivity between ACC regions and other fronto-limbic structures within both the cognitive and affective divisions would be significantly decreased among depressed adolescents with histories of suicide. This is consistent with the roles this area has in executive function,response selection,and regulation of emotion (Bush et al., 2000).

Methods and Materials

Participants

This study was part of a broader study that was approved by the University of Minnesota institutional review board (Cullen et al.,2009;2010). The original study aimed to use neuroimaging measures to examine the difference between depressed and healthy adolescents and in this secondary data analysis, 24 adolescent subjects between the ages of 15 and 19 were included in this study. This sample was comprised of 12 depressed participants (4 patients with histories of suicide attempts and 8 patients without histories of suicide attempts)and 9 healthy controls for comparison.Exclusion criteria for the original sample included an intelligence quotient (IQ) of less than 80 as determined by the Wechsler Abbreviated Scale of Intelligence (Wechsler, 1999), conditions incompatible with MRI such as claustrophobia and metal implants, significant medical or neurological disorders, and a positive urine pregnancy test for females. Further exclusion criteria for the depressed group included the following psychiatric comorbidities: schizophrenia, a pervasive developmental disorder, bipolar disorder, a substance-related disorder with use within the past 60 days, and an eating disorder with active symptoms in the past 12 months. Other common comorbidities, such as anxiety, were permitted on the condition that major depressive disorder was the primary diagnosis. Exclusion criteria for healthy controls included a past or current DSM-IV diagnosis. Both healthy controls and depressed adolescents were recruited through the use of community postings. Additionally, depressed subjects were recruited from psychiatric programs at the University of Minnesota Medical Center - Fairview Hospital.

Informed consent was obtained for those 18 and over. For those younger than 18, a parent or guardian provided written consent and the participant provided assent. All participants were compensated for the portions of the study they completed.

Clinical Assessment

DSM-IV Axis I diagnoses were assessed using the Schedule of Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (KSADS-PL) (Kaufman et al., 1997), in which separate semi-structured interviews are conducted with both parent and child. Additionally, subjects completed the Beck Depression Inventory-II (BDI-II) (Beck, Steer, & Brown,1996) to assess symptom severity and type. The Global Assessment of Functioning (DSM-IV) was assessed for each patient as well.Those in the depressed group also reported, in months, the duration of current illness.

Scan Acquisition

Because this study utilized the same sample as Klimes-Dougan et al.(under review), the following procedures are the same as what was published previously. Neuroimaging was conducted at the Center for Magnetic Resonance Research at the University of Minnesota using a Siemens Trio 3 Tesla scanner (Erlangen, Germany).Each participant completed a 6 minute resting state scan during which they were instructed to keep their eyes closed and rest. This consisted of 180 contiguous echoplanar imaging (EPI) whole brain volumes (TR=2000ms; TE=30ms; flip angle=90degrees, 34 contiguous AC-PC aligned axial slices; matrix = 64x64; FOV = 220mm; acquisition voxel size = 3.4 x 3.4 x 4mm, matrix=64). A 10 minute high-resolution T1-weighted anatomical image was also obtained using a magnetization prepared gradient echosequence(MPRAGE),TR =2530ms; TE=3.63ms; TI=1100ms; flip angle= 7 degrees; 1mm slices, FOV = 256mm, GRAPPA=2, 10 minutes. Further details of image acquisition can be found in Klimes-Dougan et al.(under review).

Data Analysis

Data Preprocessing

Data preprocessing was conducted using the following steps, which can be found in Klimes-Dougan et al. (under review).Data was visually inspected for motion related artifacts which resulted in the exclusion of a depressed participant from additional analyses due to excessive motion. Preprocessing was conducted using the FSL software package (www.fmrib.ox.ac.uk). These steps included brain extraction from the skull, high pass temporal filtration with Gaussian-weighted least squares straight line fitting, with sigma = 100s, motion correction using MCFLIRT, B0 unwarping using a field map, registration of the resting state scan to the T1 structural image using 6 degrees of freedom affine transformation, spatial smoothing (using a Gaussian kernel of FWHM 6mm), and registration to the MNI152 standard space brain using 23w degree of freedom affine transformation. Denoising was conducted using a combination of FSL and AFNI (Cox, 1996) software. AFNI was also used to identify BOLD signal outliers. Refer to Klimes-Dougan et al. (under review) for further information regarding neuroimaging preprocessing and analyses.

Statistics

A series of ANOVAs was performed to compare the fMRI means among the three groups, healthy controls, MDD subjects without histories of suicide attempts, and MDD subjects with histories of suicide attempts. Analyses were conducted on regions of interest that included connections involving the ACC. Due to the preliminary nature of this study and small sample size, statistics were conducted to assess homogeneity of variance.Post-hoc analyses were performed on significant or trending ANOVAs. Due to unequal sample sizes, we used Tukey-Kramer when equal variances could be assumed and Games-Howell for unequal variances as determined by the Levene statistic. Analyses revealing trending or significant differences between the suicidal MDD group and both the control and non-suicidal MDD groups were included in the results and discussion. Connections revealing a significant difference between the suicidal MDD group and the control group were also considered, provided that the non-suicidal MDDgroup failed to reach significance when compared to controls.

Participants

Results

All three groups were comparable in age ($M = 16.48$; $SD = 1.19$) and there was no significant difference between the non-suicidal and suicidal MDD groups in BDI scores.Overall,the sample was primarily white(76%) and female (71%). Differences in IQ among the groups were not significant($M=109.10$;$SD=10.76$).Further demographic information can be found in Table 1.

The non-suicidal and suicidal MDD groups had significantly different mean GAF scores($p=.017$).Interestingly, the non-suicidal MDD group had a lower mean GAF score($M=41$) compared to the suicidal group($M =54.25$). Mean BDI scores for the non-suicidal MDD group ($M=29.14$) and the suicidal group($M=25.25$) were not significantly different. Duration of illness was also not significant, although the suicidal group had a longer mean duration of illness ($M=6.75$months) than the non-suicidal MDD group ($M = 5.56$ months).

Resting State Connectivity

In addition to performing ANOVAs on connectivity data, one was also calculated to determine any group differences in brain volume (for information regarding volumetric measurements of this sample, refer to Klimes-Dougan et al. (under review)). Results determined that brain volume differences were not significant. Regarding connections, four were deemed to have trending significance through analyses. All of which included at least one region within the ACC. One-way ANOVAs showed trending significant differences in connectivity between the subgenual ACC and left lateral prefrontal cortex ($F(2,18)=2.77,p=.089$), the subgenual and supragenual ACC ($F(2,18)=2.63,p=.099$), the subgenual ACC and left nucleus accumbens($F(2,18) =3.08,p=.071$), and right amygdala and right rostral ACC($F(2,18)=2.35,p=.124$). Compared to controls ($M = .261$, $SD = .144$), the suicide group had red connectivity between the left lateral prefrontal cortex and subgenual ACC, which trended toward significance ($M = -.054$, $SD = .246$, $p = .091$). Such reduced activity trending toward significance was also found between the subgenual and supragenual ACC among the suicide group ($M = .072$, $SD = .226$) compared to controls ($M = .365$, $SD = .152$, $p = .122$). In these cases, differences between non-suicidal and suicidal MDD patients were not significant, although it should be noted that there were also no significant differences between the non-suicidal MDD group and controls.

The suicide group (M = .203, SD = .273) showed decreased connectivity between the subgenual ACC and left nucleus accumbens compared to both controls(M = .500, SD = .151, p = .151) and the non-suicidal MDD group (M = .582, SD = .325, p = .061), which trended toward significance. This was also found for the connection between the right amygdala and right rostral ACC as suicidal patients had lower mean connectivity(M= -.060, SD = .020) compared to controls (M = .040, SD = .094, p = .034) and non-suicidal patients (M = .038, SD = .083, p = .031) using Games-Howell post-hoc due to unequal variances.

Discussion

As hypothesized, these findings suggest the possibility of abnormal fronto-limbic neural circuitry among depressed adolescents who attempt suicide. Indeed, some of our findings are consistent with the assumption that MDD patients with suicidal histories would have similar, but more severe, neurobiological abnormalities as MDD patients without suicidal histories. This is illustrated by the reduced connectivity between the left lateral prefrontal cortex and the subgenual ACC and also the subgenual to supragenual ACC. These findings are supported by the more robust findings between the MDD patients with histories of suicide attempts and controls than the depressed non-suicide attempters and controls, which are depicted in Figure1. Previous findings of March and et al. (2012) also support the idea of suicidality as being on the severe end of a continuum of MDD symptoms.

The reduced activity involving the left lateral prefrontal cortex to the subgenual ACC, though only trending toward significance, suggests that MDD patients with histories of suicide may have difficulty utilizing executive function in the regulation of their emotions. This may lead to problems in successfully delegating the appropriate emotional response even in resting-state conditions. Furthermore, the prefrontal cortex has been implicated in emotion asymmetry, in which the right hemisphere is associated with negative affect while the left hemisphere is associated with positive affect(Davidson,1995). An early neuroimaging study by Jones and Fox(1992)found greater activation of the right hemisphere in the presence of negative affect and greater activation of the left hemisphere in the presence of positive affect. The findings of the present study suggest that adolescents with MDD and previous suicide attempts may have more of an absence of positive emotion compared to controls. However, it should be noted that there has been criticism directed toward the hypothesis of affective asymmetry (for review, see Alves, Fukusima, & Aznar-Casanova, 2008).

Although the above findings suggest that those with suicidal behaviors have similar, but possibly more severe, neurobiological differences as depressed patients without suicidal histories, we did find possible support for connections that highlight a distinction between these two groups. This is demonstrated through the findings of decreased connectivity between (1) the subgenual ACC and nucleus accumbens and (2) the right amygdala and the right rostral ACC among the MDD patients who have attempted suicide, which trended toward significance.These findings are consistent with the suggestion presented by Wagner et al.(2011)for suicidality having a distinct neurobiology compared to non-suicidal MDD patients. Furthermore, one of the regions implicated in the study by Wagner and colleagues is the rostral ACC. According to a review by Pizzagalli (2011), increased activation of the rostral ACC has been found to be a predictor of treatment success. Pizzagalli also suggested that reduced resting-state activity in the rostral ACC may be linked to an inability to use adaptive self-focused processing, which may contribute to poor treatment outcome. This may suggest that our sample of suicidal MDDpatients may be more treatment resistant than their non-suicidal counterparts and therefore, may require more intensive therapeutic techniques. Additionally, our finding of decreased connectivity between the right amygdala and right rostral ACC is somewhat consistent with the findings of Carballedo et al.(2011). However,the finding of Caballedo and colleagues was among a depressed sample in which suicidality was neither excluded nor examined as a separate entity. By examining MDD patients without histories of suicide,the present study did not replicate these findings.

Although only trending toward significance, the decreased connectivity among the suicidal MDD patients between the right amygdala and right rostral ACC as well as the subgenual ACC and left nucleus accumbens provides potential support for the dysregulation of the affective division of the ACC (Bush, Luu, & Posner, 2000). This division, as stated in the review by Bush and colleagues, is responsible for regulating emotional responses as well as determining the importance of motivational and emotional information. The decreased activity in this area among patients who have attempted suicide may suggest that these individuals may have difficulty interpreting the salience of emotional cues and may be emotionally volatile due to an inability to successfully manage their feelings. Further research using a larger sample size as well as stimuli to evoke emotionality, such as those used by Anand et al. (2005) and Carballedo et al. (2011) should be conducted to provide more support for this conclusion.

Limitations and Future Directions

A limitation of the present study is the small sample size due to its preliminary nature. Analyses should be replicated on a much larger sample of adolescents before further conclusions may be drawn. Furthermore, despite one's assumptions, depressed adolescents with suicidal histories did not differ in symptom severity as measured by the BDI-II. GAF scores also did not differ among the two groups. A larger sample size may reveal a much different pattern as one would expect those who resort to suicidal behaviors to have a more severe symptom pathology and difficulty in functioning.

Another direction for future research would be to explore suicidal thoughts and behaviors on a continuum to discover possible correlations in connectivity. A categorical approach can also be utilized, such as in the PET study by Oquendo et al.(2003), to examine the differences between individuals who have a history of high or low lethality suicide attempts. Suicide ideation combined with a desire for death can be an additional category to add to these analyses as this combination has been found to be the best predictor for lifetime suicide attempts compared to the presence of either of these alone(Baca-Garcia et al.,2011). This will be helpful in identifying what patterns are neurobiological predictors to suicide attempts rather than a possible result of the behavior.Furthermore, as mentioned previously,future fMRI research can also examine connectivity differences among these groups using emotional stimuli.

It should also be noted that although approximately 98% of those who die by suicide suffer from at least one mental illness, only 30.2% suffered from mood disorders (Bertolote, Fleischmann, DeLeo, & Wasserman, 2004). Bertolote and colleagues further report that other diagnoses include substance-use disorders (17.6%), schizophrenia (14.1%), and personality disorders (13%). Because only a relatively small percentage of those who die by suicide suffer from depression, research should also examine this phenomenon independent of a particular diagnosis. Information gathered from these studies may assist in providing clinicians with distinct biomarkers that can possibly precede suicidal behaviors.

Because of the prevalence of attempted and completed suicides among adolescents (American Academy of Child and Adolescent Psychiatry, 2008), information directed toward the prevention of such behavior is of high value and importance. Current research on MDD and suicide has outlined the implications of the functional dysregulation of the brain as it relates to emotion regulation and processing. By furthering this research, we will be able to elaborate on the existing literature by exploring the differences between adolescents who exhibit arguably a more severe MDD pathologythatinvolves suicidal thoughts and behaviors and adolescents who experience MDD who engage in less severe behavior. Because adolescence marks an important period of brain development (Giedd et al., 1999), this is an important age for intervention strategies as the plasticity of the brain may increase treatment effectiveness and longevity. Connection deficits shown may help direct appropriate treatment strategies that can target these deficiencies and thus, provide more effective interventions.

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